Blood Glucose and Corticosterone Changes Accompanying Altered Lipid Metabolism Induced by Exposure To Acceleration Stress

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In studies reported earlier (1), some marked changes in fatty acid metabolism of tissues were noted in animals exposed to acute radial acceleration stress. These changes were expressed as an increase in the conversion of radioactive acetate to lipids by slices of liver, adipose tissue, and kidney from animals exposed to stress. The magnitude of the response was related to the age of the animal, to its nutritional status with regard to feeding or withholding food, to the duration of exposure time, and to G-load.

The experimental design of this early work involves in vitro techniques on tissues removed from the animals after exposure to stress. This procedure indicates residual hormonal or blood chemical changes produced by stress. Thus, observations on changes in levels of blood glucose or plasma corticosterone levels are essential for a better understanding of some of the sequential effects of exposure. The present study correlates blood component changes with metabolic and chemical changes in the tissues.

Methods. A 10 radial-armed centrifuge having an effective operating radius of 4.5 feet was employed. The cages were mounted in the swing-bucket fashion, allowing for one degree of freedom which placed the resulting G-vector perpendicular to the cage floor. The centrifuge was illuminated with fluorescent light automatically timed to provide on and off cycling at 6:00 a.m. and 6:00 p.m., respectively.

Nine-week old Sprague-Dawley rats (225-250 g), maintained on Purina Laboratory Chow, were centrifuged at 4.7 G for varying time exposures. Noncentrifuged rats served as controls. Food was removed from the cages 24 hours prior to sacrifice. Hypophysectomized, adrenalectomized, and adrenodemedullated rats (Simonsen Laboratory, Gilroy, California) were operated on when 6 weeks of age and were kept in the laboratory for 3 weeks prior to experiment. Hypophysectomized rats were given 5% glucose for drinking purposes during the first week after surgery and thereafter, received whole-wheat bread, regular diet, and water. Adrenalectomized rats received 0.9% saline in place of water. Adrenodemedullated rats were maintained on regular diet. Plasma glucose (2) and plasma corticosterone (3) were determined on heparinized blood obtained from decapitated rats. Blood glucose (2) was determined on heparinized samples obtained from heart puncture performed under ether anesthesia.

Liver obtained from decapitated rats was cut into 0.5-mm slices with a mechanical tissue chopper (4). One gram of the slices was transferred to a 50-ml screw cap incubation flask (5) containing 10 ml of Krebs-bicarbonate buffer at pH 7.4. The medium contained 0.01 M succinate, 0.011 M glucose, and 10 μc of 0.001 M sodium acetate-1-C^{14}. Tissue was incubated for 3 hours at 37.5°C in a shaker bath in an atmosphere of 95% O_2 and 5% CO_2. The reactions were stopped by the addition of 0.2 ml of 10 N H_2SO_4 to the incubation media. The CO_2 fraction was collected with 1 ml of 2.5 N NaOH added to the removable center well. Chemical and radioisotope assays were performed as reported earlier (1).

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Results. The conversion of acetate-1-C^{14} to CO_2, nonsaponifiable lipids, and fatty acids by slices of liver from rats exposed to 4.7 G of acceleration for periods varying from 1 to 24 hours was measured. Centrifugation stress had no significant effect on the conversion of acetate to nonsaponifiable lipids or to CO_2 for any of the exposure periods studied. The effect of centrifugation on conversion of acetate to fatty acids by liver slices is shown in Fig. 1. When the data were treated by the analysis of variance, significant differences in incorporation to fatty acids were noted at 5, 8, and 24 hours. For these time periods, values for conversion of acetate-1-C^{14} to fatty acids were 0.29-0.33% per g of liver slices compared to control values of 0.10-0.20%. Fig. 1 also shows a significant lowering of fat content from the control value of 3.9% to 2.8% in liver from rats exposed to 24 hours of centrifugation.

In Fig. 2 are shown the changes occurring in blood glucose and plasma corticosterone levels as a function of exposure time. Blood glucose level rises at a half hour to a value of 240 mg/100 ml then falls to 140 mg/100 ml after 3 to 4 hours of centrifugation followed by a rise. All values for the blood glucose of experimental rats were significantly higher than values from control animals. The pattern for the plasma corticosterone curve is similar to that of blood glucose in that an initial rise to a maximum was noted from a control value of 35 μg/100 ml to a peak of 81 μg/100 ml within 1 hour after centrifugation. By the third hour after centrifugation, this level fell to a value that was found not significantly different from the control value. As centrifugation stress continued the plasma corticosterone levels rose gradually to a value of 60-65 μg/100 ml in the period of 8-24 hours.

Effects of surgical removal of pituitary, adrenal or adrenal medulla on plasma glucose, plasma corticosterone, and conversion of labeled acetate to fatty acids and CO_2 in liver slices from rats centrifuged at 4.7 G for 3 hours are shown in Table I. Centrifugation stress produced a significant increase in fatty acid synthesis in liver obtained from normal and adrenodemedullated groups. Plasma glucose levels from these two groups were also elevated following centrifugation. No response in fat synthesis was noted for the hypophysectomized or adrenalectomized rats nor was plasma glucose or plasma corticosterone found to be elevated. For noncentrifuged rats, conversion of acetate to fatty acids by liver slices from hypophysectomized rats was 0.13% which compares favorably with 0.14% for normal noncentrifuged rats. However, a depression in fat synthesis values to 0.08% was noted for adrenalectomized animals.

Centrifugation produced no alteration in conversion of acetate-1-C^{14} to CO_2 in normal or surgically-treated rats (Table I). Thus, the effects noted for conversion of acetate to fatty acids caused by exposure to centrifugation are not the result of altered substrate pool concentrations.

Discussion. Other work has shown changes in carbohydrate metabolism (6) and protein metabolism (7) in rats exposed to centrifugation for various periods of time. Oyama and Platt (6) have demonstrated increases in liver glycogen which was preceded by a rise in plasma glucose and plasma corticosterone levels. The glycogen deposition was attributed to an increased elaboration of adrenal corticosterone, as indicated by elevation of plasma corticosterone levels. Removal of the adrenal or the pituitary glands eliminated the glycogen deposition response in stressed animals. On the other hand, stimulation of liver protein synthesis induced by exposure to acceleration stress was shown to occur in adrenalectomized

or hypophysectomized rats (7). In this case, the evoked response of protein synthesis in subcellular fractions obtained from liver of rats exposed to 4.7 G for 3 hours was independent of adrenal or pituitary action.

The nature of the plasma corticosterone and blood glucose curves indicates the temporal dependence of the sequence of events following induction of the stress. The beginning of the centrifugation is followed immediately by a rise in both these blood components, resulting from the initial antagonism to the animals' intermediary metabolism. This is followed by a mobilization of body fuels resulting in deposition of liver glycogen and transport of fat presumably from adipose tissue to liver (6). At the 2-3 hour period there is an apparent reduction of supply of corticosterone or an inhibition of its release which is reflected by lowering of plasma levels. The blood glucose level is diminished at this time. As exposure continues and the animals become somewhat adapted, the blood glucose and plasma corticosterone rise. It is during this second rise that the predominant changes appear to be at the expense of altered tissue lipids as well as a changing pattern for synthesis of lipids. Thus, after 5 hours of centrifugation there is a significant increase in conversion of acetate to fatty acids with no apparent change in conversion in either CO_2 or nonsaponifiable lipids. Finally, after 24 hours of exposure to centrifugation the liver lipids have been reduced significantly in concentration. In other work (8) where the animals have been exposed to centrifugation for periods up to 15 months, the pattern for fat metabolism has changed so that the primary alteration is at the expense of conversion of acetate to nonsaponifiable lipids. After these very prolonged exposures, there is no apparent change in synthesis of fatty acids from acetate.

Removal of the pituitary gland reduced plasma corticosterone levels to near zero values and abolished the stress-induced increase in plasma glucose or fat synthesis. Absence of circulating corticosterone caused by ablation of the adrenal gland resulted in the same findings observed for hypophysectomized rats and in addition reduced hepatic lipogenesis as measured by incorporation of acetate to fatty acids. These findings suggest that the role of the adrenocortical system in regulating the hepatic lipogenetic response to centrifugation stress involves changes in supply of glucose to tissues as well as a more direct role of conversion of low-molecular weight metabolites at the level of the tricarboxcylic acid cycle to fatty acids. The findings of Abraham et al (9) that citrate-cleavage enzyme shows reduced activity in preparations obtained from livers of adrenalectomized rats lend credence to this hypothesis. Nejad and Chaikoff (10) observed reduced conversion of glucose to fatty acids in liver slices from hypophysectomized and adrenalectomized rats, but observed no reduction in conversion of acetate to fatty acids in hypophysectomized rats (11) which is a finding confirmed here.

The importance of circulating glucose as regulated by the adrenal and other endocrine glands, and the role that corticosterone plays in directly regulating the stress-induced changes in fat metabolism are presently under investigation as a continuation of this study.

Summary. Fasted, male, Sprague-Dawley rats were exposed to 4.7 G for periods of time up to 24 hours. Plasma glucose, plasma corticosterone, liver lipids, and incorporation of acetate-1-C^{14} into fatty acids in liver slices was followed in rats exposed for periods of 1-24 hours. Plasma glucose and plasma corticosterone curves were bimodal showing an early maximum during the first 3 hours of exposure and rising after 5 hours through the 24-hour study.

During the second rise the greatest changes in lipid metabolism were noted as a decrease in liver lipid and an increase in fatty acid synthesis. The responses evoked by the stress were abolished by hypophysectomy or adrenalectomy. It is concluded that changes in fat metabolism induced by acceleration stress were mediated, in part, by changes in levels of circulating glucose, corticosterone, or the interaction of both.

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TABLE I. Plasma Corticosterone, Plasma Glucose, Hepatic Lipogenesis, and Acetate Oxidation of Normal, Adrenodemedullated, Hypophysectomized, and Adrenalectomized Rats Exposed to 4.7 G for 3 Hours.

Treatment	No. of rats in group	Plasma glucose (mg/100 ml)		Plasma corticosterone (μg/100 ml)		% conversion of acetate-1-C^{14} to fatty acids per g of liver		% conversion of acetate-1-C^{14} to CO_2 per g of liver	
		Control	Centrifuged	Control	Centrifuged	Control	Centrifuged	Control	Centrifuged
Normal	8	101±1	139±5	35±2	42±4	.14±.01	.20±.03	44.6±1.8	43.5±0.9
Adrenodemedullated	8	110±2	138±3	39±2	42±3	.13±.01	.34±.08	42.6±1.8	37.6±3.3
Hypophysectomized	8	97±2	100±3	3±1	1±0	.13±.01	.14±.04	51.2±3.0	48.6±1.0
Adrenalectomized	8	93±2	102±3	1±0	2±0	.08±.00	.08±.01	42.3±1.8	44.6±1.0

1.0 g of liver, obtained from 9-week-old fasted rats, was incubated for 3 hours at 37.5°C in 10.0 ml of medium. Each value represents mean ± standard error.

Figure Titles

Fig. 1. Effect of time of exposure to 4.7 G (experimental) on total lipid content in liver and on conversion of acetate-1-C^{14} to fatty acids in liver slices from fasted 9-week-old rats exposed to 4.7 G. Each value represents mean ± SE of 8 animals.

Fig. 2. Effect of time of exposure to 4.7 G (experimental) on plasma corticosterone and blood glucose from fasted 9-week-old rats exposed to 4.7 G. Each value represents mean ± SE of 8 animals.

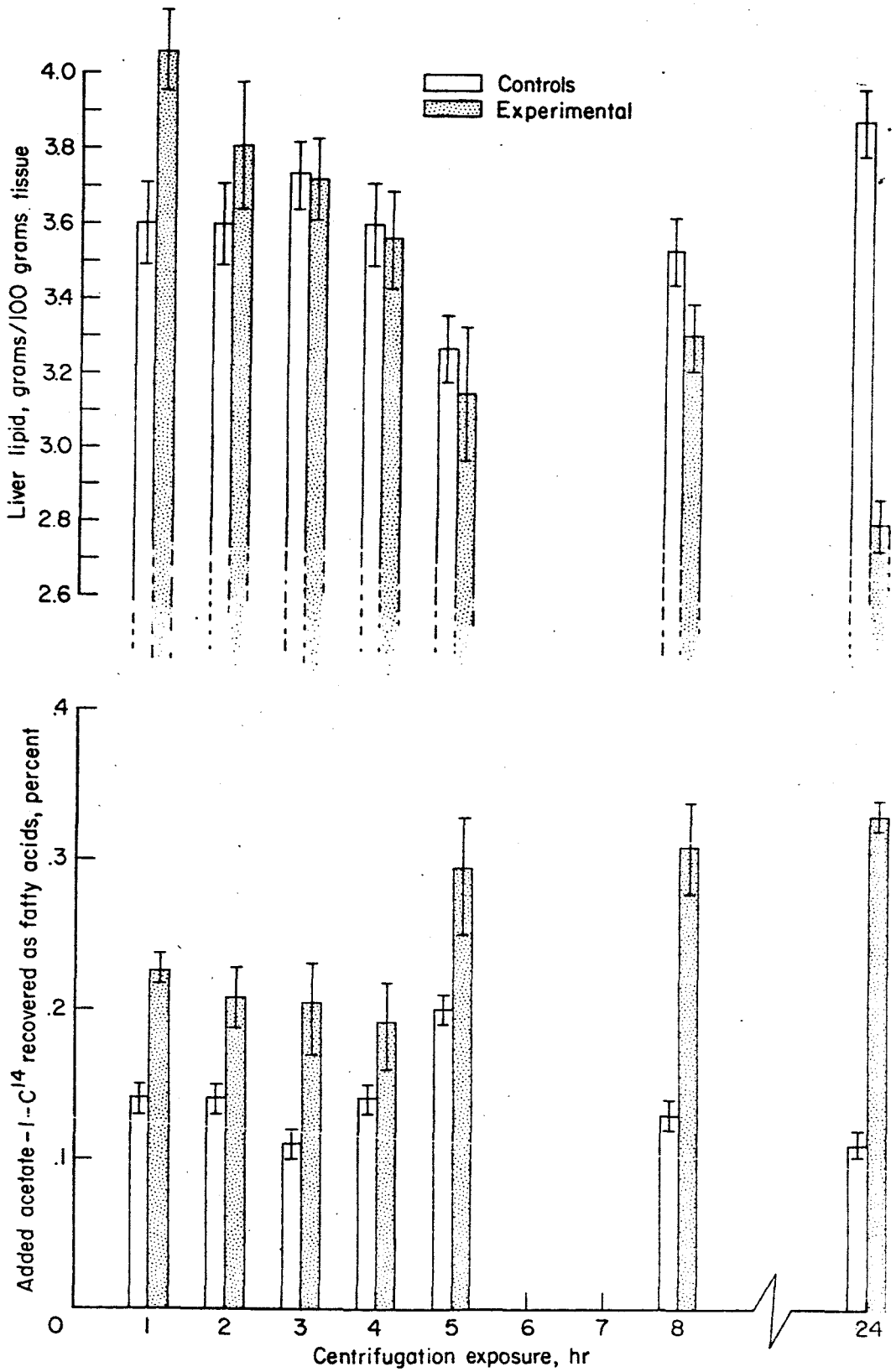

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Fig 1.

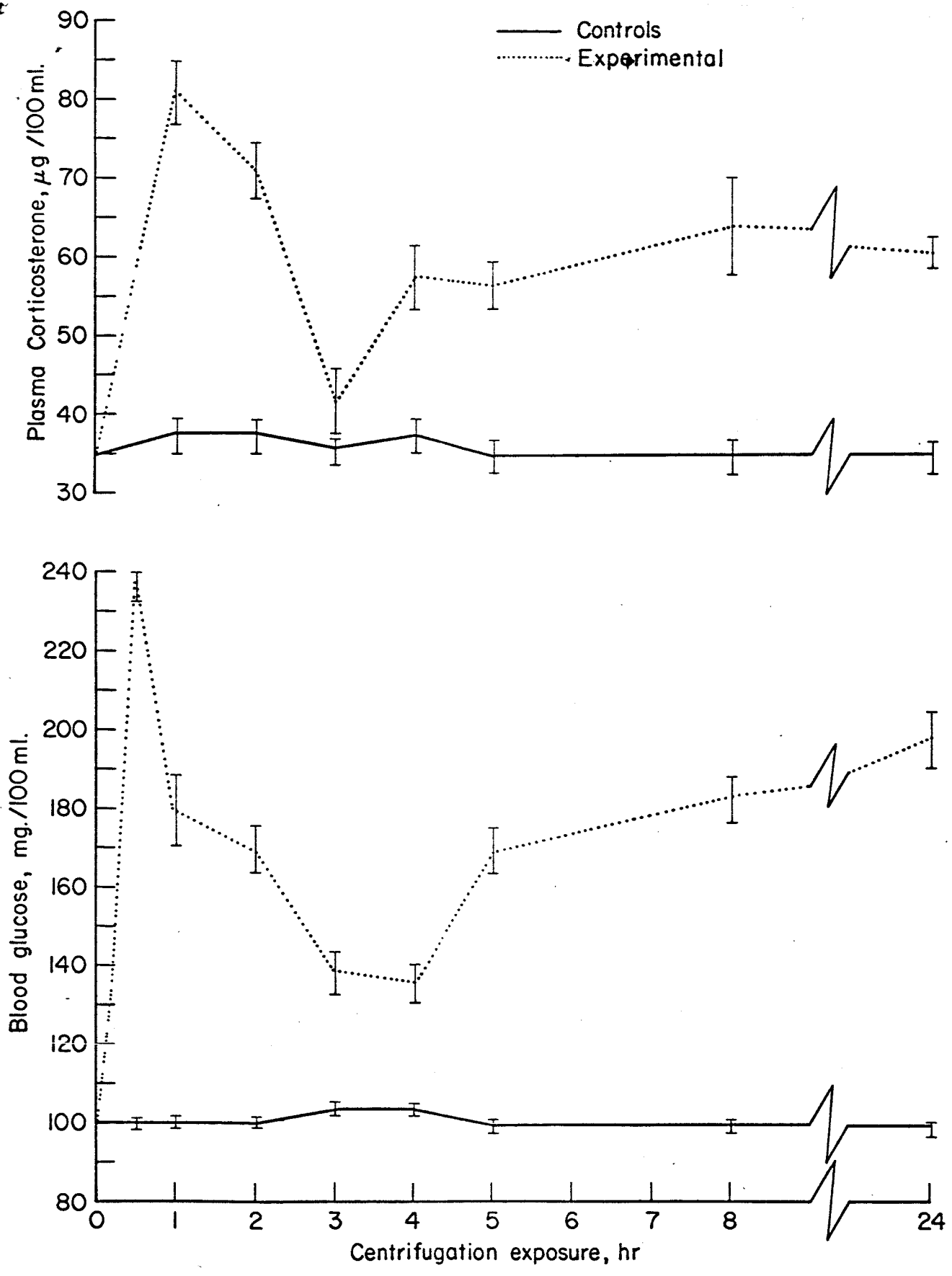

Fig. 2

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